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INFLUENCE OF ABIOTIC FACTORS ON THE GROWTH OF *Colletotrichum musae* CAUSING POST HARVEST ANTHRACNOSE DISEASE IN BANANA

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ABSTRACT

Banana (Musa spp.) is the most important fruit in many tropical and subtropical region of our country. It is affected by many post harvest diseases, among them anthracnose is considered as the most important disease of banana in the global level and is one of the major constraints to banana production. Effect of different pH, temperature and light intensity on mean mycelial growth of different isolates C. musae under in vitro was studied. The results revealed that pH 7 recorded the maximum mean mycelial growth of 88.50 mm in C5 isolate and the minimum mean mycelial growth of 49.50mm was recorded in C8 isolate. The maximum mean mycelial growth of 88.69 mm was recorded at 30°C and the lowest mean mycelial growth was recorded at 35°C (45.86mm). The results revealed that all the isolates of C. musae grew well when they were exposed to alternate cycles of 12h dark and 12h light. The highest mean mycelial growth of 82.50mm was recorded when the isolates were exposed to alternate cycles of 12h light and 12h and the lowest mean mycelial growth of 35.59mm was recorded when the isolates where exposed to 12h dark. Thus it may be concluded that the temperature, pH and light are the critical factors for the growth of pathogen in vitro, which might be the main reason for the expression of banana anthracnose symptoms under postharvest conditions.

KEYWORDS: Abiotic Factors, Banana, Colletotrichum musae, Disease & Post Harvest

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Original Article

INTRODUCTION

Banana (*Musa* spp.) is the largest herbaceous flowering plant originated from Malaysia. It belongs to the order Zingiberales and family Musaceae, with two important genera *Musa* and *Enseta*. It is one of the ancient tropical fruit crops in India and known as apple of paradise, poor man fruit and Adam's Figure (Bose and Mitra, 2001). Banana is known for its antiquity and it is interwoven with Indian heritage and culture. It is considered as the symbol of 'prosperity and fertility'. Owing to its greater socio-economic significance and multi-faceted uses, it is referred as 'kalpatharu' (plant of virtues) and kalpavriksh. Banana constituted the fourth most important global food commodity after rice, wheat and maize in terms of gross value of production. Banana being a rich source of carbohydrate, vitamin A, B-6, C and also rich in minerals like potassium, phosphorus, calcium and magnesium. It is grown in more than 150 countries. The production of banana in India was 3.56 million tonnes (FAO, 2015). The total area under banana production in Tamil Nadu is 1.18 lakhs ha with an annual production of 5.65 million tonnes (FAO, 2015).

The crop is prone to the attack of several diseases, among which Panama wilt, sigatoka leaf spot, anthracnose, bunchy top, tip over, moko wilt, banana streak virus and banana bract mosaic virus are found to be predominant. Banana being a highly perishable commodity, due to lack of awareness in the production causes heavy post harvest losses up to 35% (Albashir and Imam, 2010) during transport and storage, which deteriorates

the fruit quality very rapidly after harvest. The most common post-harvest diseases of banana are fruit rots, crown rot, finger rot and cigar end rot. (Manica, 1997). Among the post harvest diseases, banana anthracnose is considered as the most important diseases of banana in the global level and is one of the major constraints to banana production. It deteriorates the quality and nutritive value of the fruits and renders them unfit for marketing and consumption thereby causing severe loss to farmers and traders. The losses may be tune of 100 per cent if they are not managed properly (Ploetz, 1998). Anthracnose caused by *Colletotrichum musae* (Berk. and Curt) Arx. occurs in almost all banana growing countries. Primarily, anthracnose is considered as a storage disease but infections of immature fruits do occur in the field and have been reported from Australia, India, Fiji and Philippines. Among the various factors responsible for post-harvest losses in banana, especially banana anthracnose caused by *C. musae,* is a major one intended for local as well as distant markets (Jeffries *et al*., 1990). The main control measures against post harvest diseases are by applying fungicides (Thompson and Burden, 1995). The metabolic and catabolic activity of an organism varies depending on the hydrogen ion concentration existing in the surrounding environment. Hence, pH plays a role in deciding the nature and activities of microorganisms (Munro, 1970). Temperature affects the physiological function of the fungi, which in turn affect the phenotypic expression. Based on this background the current research work was carried out and results are discussed in this article.

MATERIALS AND METHODS

Collection and Isolation of Pathogens

Infected samples were collected from different markets of Coimbatore and Namakkal districts. The infected fruits showing typical dark sunken spots with salmon colour spores were collected in butter paper bags and later placed in polythene covers. The infected fruit samples were first microscopically examined to confirm the presence of fungal pathogen *Colletotrichum*. The diseased tissues were teased with a sharp blade on a glass slide having a drop of clear water and covered with cover slip to confirm the presence of fungal spores under microscope (10x, 40x). After confirming the presence of fungal spores, isolation was carried out in the laminar air flow chamber under aseptic conditions following tissue isolation method. The infected tissue of fruits which shows typical symptoms were cut in to small bits measuring about 2mm and surface sterilized in 0.1% mercuric chloride solution for one minute and washed repeatedly thrice with sterile distilled water to remove the trace of mercuric chloride. The surface sterilized tissues were transferred to sterile Petri plates containing potato dextrose agar medium under aseptic conditions. The inoculated Petri plates were incubated under room temperature (25±2°C) and observations were taken at regular growth. The pathogen was identified up to species level based on their cultural and morphological characters. Totally 11 different *C. musae* isolates (C1-C11) were isolated and investigated under *in vitro*.

Effect of Different pH on the Growth of *C. musae* Isolates

The effect of pH on the growth of the pathogen was studied as per the method followed by Kiryu (1939) using PDA medium. Different level of pH *viz.,* 5.0, 6.0, 7.0, 8.0 and 9.0 were used. The pH level was adjusted in a digital pH meter using 0.1 N Sodium hydroxide and 0.1 N Hydrochloric acid. The medium with different pH levels were sterilized cooled and poured in the petriplates in 20 ml quantities and allowed to solidify. The eight mm disc of pathogen was placed on the centre of the petri plates. The plates were incubated at room temperature (28 ± 2°C) for ten days. The diameter of the mycelial growth was recorded for all the treatments. Three replications were maintained for each treatment.

Effect of Different Temperature on the Mycelial Growth of *C. musae*

The effect of different temperatures on the growth of pathogen was studied. Different temperatures maintained for the growth of the pathogen on PDA were 15, 25 and 30°C. Mycelial disc of 8 mm was used to inoculate petriplates. Three replications were maintained for each treatment. Inoculated plates were kept in incubator and temperature was adjusted to required level. The mycelial growth was recorded at seventh day after inoculation.

Effect of Light Intensity on the Mycelial Growth of *C. musae*

The effect of light on the growth of pathogen was studied by exposing the inoculated culture to alternate cycles of 24 h light, 24 h dark and 12 h light and 12 h dark in an environment chamber maintained at room temperature (28±2°C). Mycelial disc of 8 mm was used to inoculate Petri plates. Three replications were maintained for each treatment. Inoculated plates were kept in environment chamber and light intensity was adjusted to required level. The mycelial growth was recorded at seventh day after inoculation.

Statistical Analysis

The data were statistically analyzed using the IRRISTAT version 92 developed by the International Rice Research Institute Biometrics unit, the Philippines (Gomez and Gomez, 1984). Prior to statistical analysis of variance (ANOVA) the percentage value of disease index were arcsine transformed. Data were subjected to analysis of variance (ANOVA) at two significant levels ($P<0.05$ and $P<0.01$) and means were compared by Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSIONS

Worldwide post harvest losses are estimated to 45 per cent in perishable food crops due to post harvest diseases (Aulakh *et al.*, 2013). In India, post harvest losses due to diseases, including anthracnose, have been estimated to 20 to 25 per cent or more of the post harvest produce (Singh and Sharma, 2007). Post harvest losses representing up to 25 per cent in industrialized countries and more than 50 per cent in developing countries have been reported (Nunes and Nunes, 2012). Effect of pH, temperature and light on the mycelial growth of different isolates of *C. musae* was studied. The results revealed that the maximum mean mycelial growth of 84.16 mm was observed at pH 7.0 followed by pH 6.0 (75.24mm). The lowest mean mycelial growth of 54.77 was recorded at pH 9.0. The pH below six and above seven was detrimental to the growth of pathogen (Table 1). In the present study, maximum mean mycelial growth of 84.16mm was observed at pH 7 followed by pH 6.0 (75.24mm) which is in agreement with the results of Ranjitham *et al.* (2011). They reported that the maximum growth of *C. musae* was at pH 7 followed by pH 6. However, this is similar to the work of Gina (1999) identified pH 7 is the optimum for the mycelial growth of *Colletotrichum gloeosporioides* isolated from mango and avocado. Maqsood *et al.* (2014) recorded maximum mycelial growth of *C. gloeosporioides* in pH of 6.5 followed by 6.0. At reduced pH, cell membrane becomes saturated with the hydrogen ions which limit the passage of cations. The reverse could be obtained when medium are alkaline and accumulated hydroxyl ions preventing the passage of essential anions. In addition, the enzyme activity is also conditioned by reaction of the medium, as a result the reduced growth of both fungi is observed at both the extremities (Bilgrami and Verma, 1978).

Effect of different temperature on the mycelial growth of different isolates of *C. musae* was studied. The results revealed that all the temperatures were supported the mycelial growth of the isolates *C. musae.* The highest mean mycelial growth of 88.69 mm was observed in 30°C which was followed by 25°C (81.50mm). The lowest mean mycelial growth of 45.86mm was recorded in 35°C (Table 2). Temperature is most important physical environmental factor for regulating the

growth and reproduction of fungi. The present study revealed that good growth of fungus was observed at 20–30°C. The maximum mean mycelial growth was recorded at 30°C (88.69mm) which was followed by 25°C (81.50mm). Ranjitham *et al.* (2011) observed the maximum mycelial growth of *C. musae* at 30°C (89.69mm) which was followed by 25°C (87.69mm). Prabakar (1997) reported that *C. gloeosporioides* isolated from mango recorded the maximum mycelial growth at 25°C followed by 30°C and temperature below 20°C and above 35°C were inhibitory to the growth. The temperature of 25ºC was reported to be the optimum for the growth of *C. gloeosporioides* on mango, almond and avocado (Adaskaveg and Hartin, 1997; Gonzalez, 2003; Moriwaki *et al*., 2003). Maqsood *et al.* (2014) recorded the maximum growth of *C. gloeosporioides* which was isolated from mango incubated at 28°C (8.83cm) followed by 30°C (8.2 cm) and minimum growth (5.40 cm) was obtained when incubated at 28°C. Nandinidevi (2008) reported that maximum growth of *C. gloeosporioides* at 25°C followed by 30°C which was isolated from anthurium.

CONLUSIONS

Effect of light intensity on the mycelial growth of different isolates of *C. musae* was studied. The results revealed that all the isolates grew well when they were exposed with alternate cycles of 12 h dark and 12 h light with mean mycelial growth of 82.50mm followed by 24 h light exposure (56.41mm) and the lowest growth of all the isolates was found when exposed to 24 h dark (35.59mm) (Figure 1). Light has profound effect on the mycelial growth of *C. musae*. The present study, maximum mycelial growth was observed when it was exposed to alternate cycles of light and darkness (82.50mm). This was followed by continuous light (56.41mm) and continuous darkness (35.59) Ranjitham *et al.*(2011) reported that all isolates of *C. musae* grew well when they were exposed with alternate cycles of 12 h dark and 12 h light with mean mycelial growth (87.32 mm) followed by 24 h light exposure (56.20 mm) and the lowest growth of all isolates was found when exposed to 24 h dark (37.58 mm). Maqsood *et al.* (2014) observed that *C. gloeosporioides* isolated from mango grew best at the exposure of 12 hr light and 12 hr darkness followed by 24 hr light exposure whereas, their growth was found at minimum at the exposure of 24 dark. Kamanna (1996) found that *C. gloeosporioides* isolated from coffee, when exposed to alternate cycle of 12h light and 12 h dark yielded maximum growth of when compared to the continuous exposure of light or dark. Kanappa (1998) showed that exposure of coffee anthracnose pathogen *C. gloeosporioides* to alternate cycles of 12 hr light and 12 hr darkness yielded maximum growth and sporulation. Similar results were recorded by Alexander *et al*. (2004) in *C. gloeosporioides* isolated from green pepper. The exposure of stylosanthes anthracnose pathogen *C. gloeosporioides* to alternate cycles of light and darkness showed maximum growth and sporulation (Sudhakar, 2000). These reports supported the results of the present study. Fungi generally utilize substrates in the form of solution only if the reaction of solution is conducive to fungal growth and metabolism. This brings importance of hydrogen ion concentration for better fungal growth.

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APPENDICES

Table 1: Effect of Different pH on the Mycelial Growth of *C. musae* Isolates

Isolates	Mycelial Growth (mm)				
	5.0	6.0	7.0	8.0	9.0
C1	67.70^{e}	72.40^{d}	82.25^{ab}	62.50^{f}	55.00^{g}
C2	65.00^{e}	77.50^{c}	80.00^{c}	64.50^{f}	51.50^{h}
C3	62.50^{f}	76.00^{c}	81.50^{ab}	65.50^{e}	52.50^{h}
C4	61.00^{f}	78.50^{c}	86.50^{a}	61.50^{f}	50.00^{h}
C5	69.50^{e}	81.25^{ab}	88.50^{a}	72.00^{e}	62.50^{f}
C6	63.25^{f}	75.00^{c}	85.50^{ab}	63.00^{f}	56.00^{g}
C7	68.50^{e}	80.50^{c}	85.00^{ab}	66.00^{e}	54.00^{h}
C8	60.00^{f}	69.0^{e}	78.50^{c}	58.00^{g}	49.50^{i}
C9	67.50^{e}	72.50^{d}	87.50^{a}	63.50^{f}	57.00^{g}
C10	65.50^{e}	71.50^{d}	84.00^{ab}	62.00^{f}	56.50^{g}
C11	63.50^{f}	73.50^{d}	86.50^{a}	69.00^{e}	58.00^{g}
Mean	**64.90**	**75.24**	**84.16**	**64.32**	**54.77**

Mean of three replications

In a column, means followed a common letter is not significantly different at the 5% level by DMRT

Table 2: Effect of Different Temperature on the Mycelial Growth of *C. musae* Isolates

Isolates	Mycelial Growth in mm				
	15°C	20°C	25°C	30°C	35°C
C1	53.50^{g}	65.00^{e}	85.00^{ab}	90.00^{a}	46.50^{h}
C2	57.00^{f}	79.00^{d}	90.00^{a}	89.00^{a}	42.50^{i}
C3	55.00^{f}	75.00^{d}	82.00^{ab}	88.50^{a}	47.00^{h}
C4	51.50^{g}	74.00^{d}	84.00^{ab}	90.00^{a}	48.00^{h}
C5	70.50^{e}	85.50^{ab}	90.00^{a}	86.50^{ab}	49.50^{h}
C6	69.00^{e}	80.00^{c}	90.00^{a}	90.00^{a}	46.50^{h}
C7	49.00^{g}	62.00^{f}	82.00^{ab}	89.50^{a}	42.00^{i}
C8	56.50^{f}	55.00^{f}	67.00^{e}	85.56^{ab}	40.00^{i}
C9	52.50^{g}	73.00^{d}	86.50^{ab}	89.00^{a}	48.50^{h}
C10	68.00^{e}	75.00^{d}	75.00^{d}	87.50^{a}	47.50^{h}
C11	60.00^{f}	85.00^{ab}	85.00^{ab}	90.00^{a}	46.50^{h}
Mean	**58.41**	**73.50**	**81.50**	**88.69**	**45.86**

Mean of three replications

In a column, means followed a common letter is not significantly different at the 5% level by DMRT

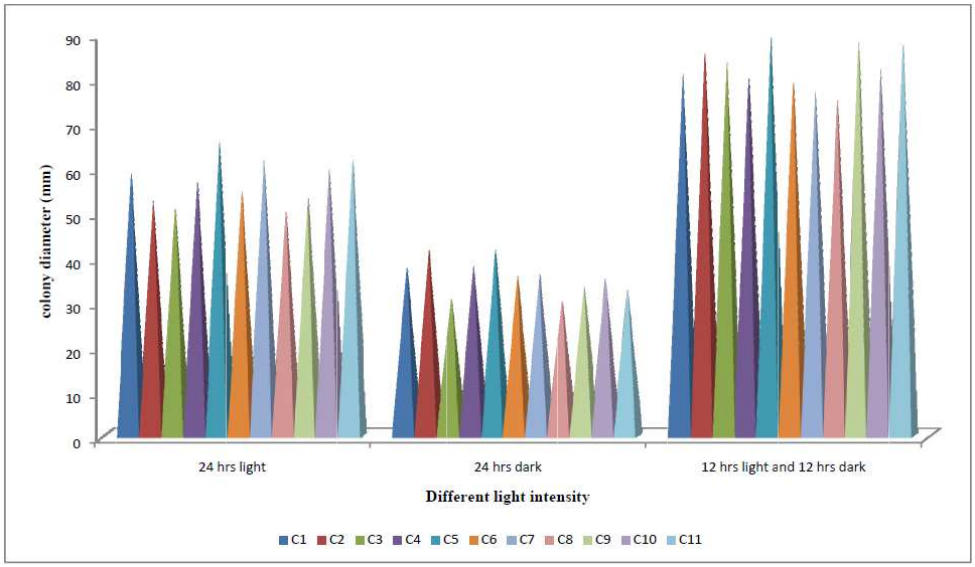

Figure 1: Effect of Light Intensity on the Mycelial Growth of *C. musae* Isolates